

THE MALE SEX HORMONE

SOME FACTORS CONTROLLING ITS PRODUCTION AND SOME OF ITS EFFECTS ON THE REPRODUCTIVE ORGANS

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That the testis has a function other than the production of spermatozoa has been known for many years. Indeed, the first experimental demonstration of endocrine activity for any organ occurred in 1849 when Berthold transplanted testes in the male fowl and observed that the usual external effects of castration did not occur in the bird bearing a transplanted gonad. Berthold's work has been confirmed repeatedly and, indeed, the effect of testicular hormone on the comb is at this time the basis of the principle method employed in gauging the activity of male hormone preparations. Complete accounts of the effects of testicular hormone upon the plumage and head furnishings of birds are given by Moore (1932) and Domm, Gustavson and Juhn (1932). Moore (1932) Witschi (1932) and Willier (1932) have adequately considered the question for lower animal forms as well as the embryological relations.

Berthold's observations on the relation of the testis to secondary sex characters in the fowl were eventually applied to mammals and it is now known that the normal function of the sex-accessory organs, viz., the seminal vesicle, prostate, epididymis, and Cowper's glands, depend upon the internal secretion of the testis. Furthermore, there is excellent evidence for the existence of a testis-hormone control over physiological maturation of the spermatozoa. Morphologically, the sperm are, apparently at least, mature when they are released from the testis into the epididymis. However, such sperm have a low capacity for fertilization. After a period of time spent in the epididymis under the influence of the male hormone their fertility is enhanced. This period of residence in the epididymis is looked upon as one during which physiological maturation occurs.

We, therefore, recognize the profound influence exerted over a group of reproductive organs by the internal secretion of the testis. However the testis itself is under the immediate control

of another endocrine organ, the anterior pituitary. This minute gland is believed to secrete two hormones which influence the activity of the male gonad. One of these is concerned with the stimulation of spermatogenesis and seems to be identical with the hormone that effects follicular growth in the ovary. The identity of the second hormone, which is involved in the stimulation of the interstitial cells and consequently with the production of male hormone, is less certain. Wallen-Lawrence, and Hisaw and his collaborators have presented evidence that would associate it with the pituitary hormone which stimulates luteinization in the ovary. More recently Evans, Simpson and Pencharz have suggested that a separate hormone, the interstitial cell-stimulating hormone, is the active factor. Regardless of which of these is the hormone actually involved in the stimulation of the interstitial cells it is possible to induce, selectively, either spermatogenesis or male hormone secretion in suitable test animals. The subject used in these studies is usually the hypophysectomized rat since the action of the hormone in question may be studied in the absence of any complications which might be introduced by the animal's own pituitary.

There are other influences which are important in normal testicular function. Such dietary factors as deficiencies in Vitamines A and E and in the mineral manganese have all been shown to result in sterility. The male hormone secretion of the testis is apparently not appreciably altered by these deficiencies since the sex-accessory organs have been reported by most workers to be normal. A somewhat different condition is observed in rats on a vitamine B-deficient or an inanition diet. Such animals have testes which show apparently normal spermatogenic activity, but which have a damaged endocrine function.

Thyroidectomy in young animals impairs both the spermatogenic and endocrine functions of the testis. The damage is much less severe in the adult animal. Hyperthyroidism, induced by the repeated administration of thyroid substance, results in a profound decrease in testis size. Microscopical examination of the sex organs of hyperthyroid animals reveals the loss of both the spermatogenic and endocrine functions of the testis. The exact mechanism involved in the testis damage induced by either hypo- or hyperthyroidism is not known, but it seems probable that the anterior pituitary is involved since profound histological changes are present in the hypophyses of such

animals. In the case of hyperthyroidism the possibility that the increased metabolic rate is a deterrent to normal testicular function cannot be disregarded. Febrile conditions, generally, damage the testis. The factor involved here probably is the increased temperature to which the testis is subjected.

Related to the condition seen in the testes of animals in febrile states is the histological picture observed in the cryptorchid testis. Observations dating back to Sir Astley Cooper in 1830 and more recently emphasized by Crew and Moore have indicated that the scrotum, through its capacity for heat loss, exercises an important influence in mammalian spermatogenesis. The lower, by several degrees Centigrade, temperature of the scrotum is exceedingly important for normal spermatogenesis in mammals (whales, rhinoceroses, elephants, and cetaceans are exceptions). Thus failure of the testis to descend into the scrotum from the abdomen or its replacement, by surgical procedures, into the abdomen does not permit the production of sperm. If a testis which is producing sperm is confined by surgical procedures spermatogenesis is halted. The degenerative changes which occur are in the inverse order of spermatogenesis, i. e., the spermatozoa are lost first with the spermatids, spermatocytes, and spermatogonia following in that order. The spermatogonia are relatively very resistant and it is usually very difficult to be certain that a few are not present even after many months. Perhaps the only procedure that will give definite evidence concerning the survival of spermatogonia is the replacement of the testis in the scrotum. Surviving germ cells will permit the resumption of spermatogenic activity.

It is commonly supposed that only the germinal elements of the testis are impaired when the organ is subjected to the condition of cryptorchidy. Thus it is said, e. g., Moore (1932) that the endocrine function of the testis is quite normal. This observation is unquestionably true in instances where the cryptorchid condition has existed for a relatively short time. Most observations on the rat have continued for only a few months and as far as the writer knows none have been made in cases of more than six months. In the guinea pig, the other animal commonly used in these studies, one year has been the maximum. In man the reports on cryptorchid testes, naturally occurring cases, of course, are uniformly lacking in precise evidence regarding the condition of the accessories.

This question first aroused the interest of the writer during

an investigation of the hormonal control exerted over the pituitary by the testis. It had been claimed by Mottram and Cramer (1923) and more recently by McCullagh (1932) that the germinal epithelium produces a hormone that has nothing to do with the stimulation of the sex-accessories, but which is concerned with the control of the hypophysis. This control, briefly, is essentially an inhibitory effect. Thus, removal of the gonad is followed by a condition of hyperactivity in the anterior pituitary. In animals whose testes are damaged by cryptorchidism, x-ray, or vitamin deficiency the hypophyses show much the same reaction as after castration. Inasmuch as the sex-accessories of such animals are normal it was reasoned that the testis-hormone which is concerned in the activation of the accessory organs does not affect the hypophysis. It followed, with this line of reasoning, that the testis produces a different hormone and, since the spermatogenic elements are damaged by the various treatments mentioned above, it seemed logical to suppose that this second hormone is produced in the seminiferous tubules. McCullagh's experiments led him to postulate that this hormone is a water-soluble substance, in contrast to the fat-soluble hormone which stimulates the sex-accessories. On the other hand, the writer had been able to show that the fat-soluble hormone does exert a controlling effect on the hypophysis (Nelson and Gallagher, 1935). It was observed, however, that the quantity of hormone required to achieve the end-point was considerably greater than the amount necessary for maintenance of the sex-accessories. These findings strongly urged the possibility that a single testis-hormone might be concerned in the control of hypophysis and sex-accessories, and suggested that perhaps a more exhaustive study of the cryptorchid animal, than had hitherto been made, should be undertaken.

Accordingly, a large group of young male rats were set aside for study. Eighty-seven of these were made cryptorchid and sixty-nine were castrated. All operations were made between the ages of 39 and 44 days. Fifty-eight males selected from the same litters as the above animals were set aside as untreated controls. Later an additional thirty-six males were made cryptorchid. No castrate or unoperated controls were included in this series.

At intervals of about 30 days representatives of three groups were sacrificed and the pituitaries, testes, seminal vesicles and prostates were removed, weighed and prepared for study. Thus

animals were sacrificed at intervals from the thirtieth to the five hundred and tenth day after operation. The number of animals sacrificed at each stage varied somewhat, but in general there were three or four cryptorchid, three castrate, and three normal animals. The occasional death of experimental animals during the long period of time covered by the experiment added cases which did not fit into the regular intervals of the experiment. Table 1 shows the data collected on the sex-accessories of the cryptorchid animals together with a part of the data for the castrate and normal animals. It will be observed that the seminal vesicles of the cryptorchid animals continued to develop normally for about six months after operation. In the animals killed after seven months the size of the seminal vesicles was slightly decreased, but the difference is not marked. However, after 240 days there was a marked decrease in the size of the seminal vesicles, and the beginning of histological evidence for a decreased male hormone effect. During the succeeding months this became progressively more apparent until the size of the seminal vesicles was only slightly larger than that of castrates and the histological pattern was not distinguishable from the castrate. The prostate gland retained a normal size and structure until about 400 days after operation. After 500 days the size of the prostate was markedly decreased, but still considerably above the castrate level. Histologically the gland showed evidence of a lowered male hormone effect.

The fact that the seminal vesicles show "castration effects," i. e., a lowered male hormone level, in advance of the prostate is not surprising since it has been shown that the seminal vesicles require from 2 to 3 times as much male hormone as the prostates to prevent or correct the changes induced by gonadectomy. Of further interest are the observations that were made on the hypophyses of the animals in the cryptorchid series. Changes which closely simulated those occurring in the pituitaries of castrate animals, but appearing later than in the castrate controls, were present. After 75 days the "castration changes" in the pituitaries were marked enough to definitely indicate a lowered output of hormone by the cryptorchid testis. It had been shown in earlier studies that the hypophysis required about twice as much male hormones as the seminal vesicle to prevent castration changes. Thus the three organs which were used to gauge the production of male hormone by the cryptorchid testis showed castration changes in the following order: pituitary,

seminal vesicle, prostate. Furthermore, as has been noted, this order corresponds exactly with the relative requirements of these organs for male hormone. Since the hormone requirement of the pituitary is very high it is not surprising that it should be the first to show castration changes in an animal whose testis-hormone production is decreasing. It is to be expected that the seminal vesicle would show changes later than the pituitary, but in advance of the prostate whose hormone requirement is relatively low.

In addition to demonstrating for the first time that the cryptorchid testis does not maintain a normal output of male hormone this experiment seems to indicate that it is quite unnecessary to postulate the existence of a separate hormone, produced in the seminiferous tubules, which controls the anterior hypophysis. It seems reasonable to explain the harmonic control exerted by the testis over the several organs on the basis of a single hormone. In the light of our present knowledge this hormone probably is testosterone. In view of the many allied substances which qualitatively resemble the activity of testosterone it would be unwise to assume that the mother substance, e. g., testosterone, circulates in the blood unchanged and provides the necessary effect upon the hypophysis and the sex-accessories. It is quite possible that the mother hormone undergoes some change before it acts upon any one or all of the end-organs. The major point to be emphasized here is the fact that the hormone active in the control of the pituitary as well as the sex-accessories is a sterol, not a water-soluble substance.

Although the testes of the long-time cryptorchid rat are not producing sufficient male hormone to maintain a normal pituitary or sex-accessories it is possible to stimulate them to do so. In a series of 25 males which had been cryptorchid for 360 to 450 days each animal was operated upon for the removal of one testis, one seminal vesicle and a piece of prostate. The condition of these tissues was the same as has been previously outlined. These animals were then injected daily with 50 rat units of Antuitrin-S (an extract of pregnancy urine containing gonad-stimulating hormone) for 10, 20, or 30 days. At the end of the experimental period the animals were sacrificed and the hypophyses, the remaining testis and seminal vesicle, and the prostate were removed for study. The results of this experiment were striking. In all animals the seminal vesicles and prostates removed after the period of treatment were histologically nor-

mal and were as large or larger than normal. In many instances the seminal vesicle removed after the period of treatment was ten times as heavy as the one removed for control purposes. The pituitaries also showed the effect of an increased output of testis-hormone. The castration changes, which in the 360-450 day cryptorchid animal are very marked, were completely corrected in the animals treated for 30 days. The testes obtained after the period of treatment were slightly heavier than the control testes. Histological study showed that this was due entirely to a slight increase in the size and number of interstitial cells. In view of the marked increase in male-hormone production it was surprising to find relatively little change in the interstitial cells.

The fact that the hormone production of the cryptorchid testis may be stimulated by gonadotropic hormone suggests that the hypophysis of the cryptorchid animal is basically at fault. This is surprising in view of the fact that it has been shown by a number of workers that hypophyses from cryptorchid animals contain more gonadotropic hormone than do hypophyses from normal males. These results were obtained with animals which had been cryptorchid for relatively short periods of time and may not apply for cases of longer duration. The results of the experiments recounted here suggest that such is probably the case and they urge the re-examination of the accepted idea that the presence of many enlarged and vacuolated basophiles is always indicative of increased gonadotropic activity of the hypophysis.

We have described the important relation between the hypophyseal gonadotropic hormones and the testis. There can be no doubt that normal testicular function is dependent, basically, upon the hypophysis. There is, however, an accumulating body of evidence which indicates that the testicular hormone itself plays some role in the production of sperm. The first suggestion of this relationship appeared in the report of Walsh, Cuyler and McCullagh (1934) that crude extracts of male hormone would not only maintain the sex-accessories of hypophysectomized males, but would also maintain the testes in an entirely normal condition. This amazing finding was finally confirmed by Nelson and Gallagher (1936) using extracts of male hormone prepared from human urine. It was shown, however, that only the spermatogenic elements of the testis were maintained in a normal condition. The atrophic changes

which occur in the interstitial cells after hypophysectomy were not prevented. At first consideration it would appear that the physiological dictum, which states that the secretion of a gland has no stimulating effect on that gland, is distinctly violated by

TABLE I
CRYPTORCHID MALES
(Figures are Averages)

No. of Animals	Experimental Period	Body Weight (gms.)	Seminal Vesicles (gms.)	Ventral Prostate (gms.)
3	30	198	.529	.279
3	64	228	.765	.374
2	73	247	1.087	.428
3	84	243	1.005	.419
4	96	259	1.102	.436
3	120	278	1.040	.460
3	148	287	.943	.402
3	182	298	.968	.437
3	210	332	.877	.422
5	240	347	.354	.398
5	273	342	.153	.377
3	300	357	.125	.394
3	330	345	.097	.349
4	360	367	.119	.327
3	394	363	.086	.293
3	421	341	.091	.174
4	450	369	.075	.117
4	482	374	.052	.122
3	510	343	.057	.109
CASTRATE MALES				
3	30	175	.041	.052
4	96	238	.024	.042
3	240	303	.026	.037
3	300	328	.019	.033
3	510	323	.021	.035
NORMAL MALES				
3	30	217	.576	.268
2	96	295	.737	.345
3	240	378	.1216	.478
3	300	406	.1375	.462
3	510	427	1.284	.484

the results reported above. However, this is only an apparent violation if one remembers that the male-hormone is produced by the interstitial cells and that the latter have not been stimulated by the androgenic hormone, at least in the amounts thus far employed. Table 1 summarizes the pertinent data on these

observations. It should be noted that injections of male hormone must be initiated within 12 days after hypophysectomy if spermatogenesis is to be maintained. In instances where animals were allowed to remain untreated for longer periods spermatogenesis ceased or if treatment was postponed for 3 or 4 weeks it was not possible to reinitiate spermatogenesis.

In addition to confirming the original observations of Walsh, Cuyler and McCullagh it has been possible to show that male hormone extracts will maintain normal spermatogenic activity in the testis of the hypophysectomized animal for as long as 60 days. These males have marked libido and in a number of instances have sired normal litters. This is of considerable importance since it definitely proves that the sperm which are produced after hypophysectomy are quite normal. Furthermore this observation sheds light upon the question of the control of libido in the male animal. Since libido is lost more rapidly after hypophysectomy than after castration it has been supposed that the male hormone has little to do with libido. The evidence related here renders that supposition open to question since male hormone, in the entire absence of the hypophysis, will restore libido. A more reasonable explanation for the rapid loss of libido after hypophysectomy would be based upon the more serious consequences attendant upon ablation of the pituitary.

Although the very nature of the procedure employed in preparing the extracts almost definitely eliminated the possibility that gonadotropic hormone had been present in the extracts, and consequently have been responsible for the maintenance of spermatogenesis, we made a study of the effect of the material on the immature rat ovary. Even in very large dosage no stimulating effect could be demonstrated.

The recent availability of the male hormone in crystalline form permitted a further check of the absence of a pituitary effect as contributing to the results observed with the urine concentrates. Furthermore, it was hoped that the use of crystalline androgenic substances in the hypophysectomized male would be of value in elucidating the nature of the action of the male hormone on the testis. The results with the urine concentrates had suggested that the explanation might be found in the fact that the scrotum of the hypophysectomized male injected with male hormone is maintained in a normal condition while the scrotum of the untreated control regresses. It seemed possible that this regression of the scrotum might be an impor-

tant factor in the atrophy of the testes since the latter are apparently forced into the inguinal canal and finally into the abdomen. The higher temperature of the abdomen would possibly contribute to the cessation of spermatogenesis and might be the major factor responsible for it as it is in the case of cryptorchid testes. Since the testes of animals treated with male hormone are retained in scrota which appear normal it seemed reasonable to entertain the theory that scrotal maintenance might be responsible for the continuation of spermatogenesis in the absence of the pituitary. Although this idea appeared to be a satisfactory and simple explanation for the observations on animals treated with the urine concentrates the results with the crystalline androgens do not support it.

The crystalline androgens used in this study were testosterone, androsterone, dehydroandrosterone, androstenedione, androstenediol (trans), androstenediol (cis) and androstenedione. All of them were prepared synthetically.

Testosterone is the most potent androgenic substance that has been prepared. It was first obtained from testis tissue and is supposed to be the true testicular hormone.

Androsterone occurs in urine and probably was the active substance in the urine concentrates employed in the earlier studies. It is prepared from cholesterol and has about one-tenth the androgenic activity of testosterone. Androsterone is used as the international standard for the comparison of the androgenic activity of crystalline substances.

Dehydroandrosterone is also found in human urine. It has been synthesized and has about one-half the activity of androsterone.

Androstenedione has not been found to occur naturally, but has been synthesized from androsterone. It has slightly less androgenic activity than androsterone.

Androstenediol (trans) and androstenediol (cis) do not occur naturally but are synthesized from testosterone. The former has a lower androgenic potency than dehydroandrosterone while the latter Androstenedione does not occur naturally but is prepared from dehydroandrosterone. It has about the same activity as androsterone. For a complete discussion of these and other androgenic substances the reader is referred to the recent review by Koch (1937).

In the present study of the action of these substances the routine procedure adopted for their administration was the

TABLE II
HYPOPHYSECTOMIZED MALES TREATED WITH ANDROGENIC HORMONES
(Weights are given as averages)

No. OF RATS	BODY WEIGHTS		HYP. PERIOD DAYS	TREATMENT			ORGAN WEIGHTS (gms.)			SPERM MOTILITY
	First	Last		Period Days	Hormone (daily dose)	Testes	Seminal (full)	Vesicles (empty)	Prostate	
Testosterone										
3	229	195	23-24	20-21	2 mg.	1.287	2.982	.877	.733	Exc.
4	275	232	23-24	20-21	1 mg.	1.243	1.815	.651	.472	Exc.
5	243	203	23-24	20-21	0.5 mg.	1.158	1.464	.519	.463	V. good
Androsterone										
3	272	230	23-24	20-21	2.5 mg.	2.214	1.609	.529	.483	Exc.
3	281	237	23-24	20-21	1.5 mg.	1.882	1.159	.436	.365	Exc.
3	238	197	23-24	20-21	1.0 mg.	1.785	.958	.363	.277	V. good
2	226	185	23	20	0.5 mg.	1.963	.498	.355	.220	V. good
Dehydroandrosterone										
3	245	210	23-24	20-21	3.0 mg.	2.120	1.145	.443	.454	Exc.
2	242	212	23-24	20-21	2.0 mg.	2.019	.859	.335	.359	Exc.
3	239	210	23	20	1.0 mg.	1.789	.627	.295	.276	Exc.
2	242	213	24	21	0.5 mg.	1.317163	.075	Good
Androstanedione										
5	276	219	23-24	20-21	1.5 mg.	2.379	1.529	.442	.517	Exc.
2	244	197	23-24	20-21	1.0 mg.	2.117	1.297	.375	.415	Exc.
3	248	207	23	20	0.5 mg.	2.141	.350	.206	.170	Exc.
2	237	208	23	20	0.25 mg.	1.221	.209	.175	.137	Good
1	290	247	23	20	0.125 mg.	.899139	.073	No Mot.
Androstenedione										
2	228	177	23	20	1.0 mg.	2.016	1.241	.460	.515	Exc.
Androstenediol (cis)										
2	239	196	23	20	1.0 mg.	1.790	.497	.312	.215	Exc.
Androstenediol (trans)										
2	225	185	23	20	1.0 mg.	.750	.142	.113	.084	No Mot.
Controls										
12	278	217	22-24527083	.054	No Sp.
Normals										
21	267	2.432	.963	.281	.295	Exc.

solution of the materials in peanut oil so that the desired daily amount was given in one-half cc. The animals used were adult male rats four or five months of age. Injections were begun on the second day after hypophysectomy and were continued for 20 days. Table 2 summarizes the pertinent data in these experiments.

It will be noted that although all of the synthetic materials with the exception of androstenediol (trans) were active in maintaining spermatogenesis, testosterone, which is the most potent of the androgenic substances is the poorest, with the exception of androstenediol (trans), in spermatogenic effect.

TABLE III

COMPARISON OF ANDROGENIC SUBSTANCES ON THE BASIS OF THE VARIOUS TESTS

	Testes	Sex- Accessory Hypophy- sectomized Rat	Castrated Rat	Comb- Growth
Androstanedione.....	1	2	4	4
Androstenedione.....	2	3	2	3
Androstenediol (cis).....	3	6	5	6
Dehydroandrosterone.....	4	5	6	5
Androsterone.....	5	4	3	2
Testosterone.....	6	1	1	1
Androstenediol (trans).....	7	7	7	7

Androstanedione, a relatively weak androgen is the most active of the substances studied in maintaining the testis. This same lack of relationship between the potency in stimulating the sex-accessories and in maintaining spermatogenesis was shown by the other substances. Table 3 presents the rating for these compounds in terms of their capacity to maintain the testes in hypophysectomized rats and, for the sake of comparison, their potency values in the comb growth and sex-accessory tests. It is evident that the varying action of the different androgenic substances upon the testes cannot be ascribed to their potency in terms of male hormone activity. Nor is it possible to explain the different effects upon the basis of variations in the structural make-up of the substances. Androstanedione and androsterone are saturated substances while the others are unsaturated, i. e., they have a double bond in their structure. Most of the substances have a ketone and a hydroxyl group in their structural constitution although their positions may vary. There is no relation between position of these radicals and the potency of

the compounds. Perhaps the most reasonable relationship between structural constitution and potency, insofar as maintenance of the testis is concerned, is found in the fact that the two most active substances, e. g., androstanedione and androstenedione have two ketone groups. However, dehydroandrosterone and androstenedial are very active substances and they have a ketone and a hydroxyl group, and two hydroxyl groups, respectively. This same lack of correspondence between structure and testis-maintaining potency has been observed in attempts to relate structure to androgenic, i. e., comb-growth or sex-accessory stimulation, potency.

It will be recalled that spermatogenesis could not be revived in rats hypophysectomized for several weeks with the urine concentrates. A similar failure was observed when the crystalline androgens were employed.

Attention is once more called to the tentative explanation, based upon the study of animals treated with urine concentrates, for the testis-maintaining effect of male hormone. This idea was, briefly, that the well-developed scrota of the treated animals was important in enabling the testes to remain in the scrotum and consequently in maintaining spermatogenesis. While the earlier evidence distinctly favored this interpretation the more recent observations on animals treated with the crystalline androgens have caused us to abandon the idea. These results have emphasized the fact that the scrotum is definitely stimulated by androgenic hormone. Others (Hamilton, 1936, and Cutuly, McCullagh and Cutuly, (1937) have also noted this and, indeed, have advanced the idea, noted above, to explain the effect of androgens on the testis. The scrotal stimulating effect of the androgens can be shown to be proportional to the androgenic activity of the substance under test. On this basis testosterone should be, and actually is, the most effective scrotal stimulating substance. Animals injected with it, even in doses as low as 0.25 mgm. daily have very large and pendant scrota, yet their testes are not only poorly maintained, but indeed, with the lower doses they are almost as atrophic as the testes of untreated hypophysectomized animals; yet the testes of the testosterone treated animals remain in the scrotum while those of the untreated animals are abdominal or in the inguinal canal. On the other hand low doses of dehydroandrosterone or androstanedione, e. g., 0.5 mgm. daily will maintain quite satisfactory spermatogenesis yet the sex-accessories were only

slightly stimulated and the scrota, although fairly well maintained, were definitely smaller than the scrota of testosterone-injected animals whose testes were markedly atrophied.

The lack of relationship between the androgenic potency and the capacity to maintain spermatogenesis of the crystalline substances is further portrayed by the observation that extracts of the corpus luteum will maintain spermatogenesis in hypophysectomized animals. The hormone of the corpus luteum is progesterone, a substance which is closely allied structurally to the androgenic substances. It is known that unpurified extracts contain, in addition to progesterone, a substance, epi-*allo-pregnane-ol-one*, which has a relatively low androgenic activity. That this substance probably was responsible for most of the effect obtained with corpus luteum extracts is shown by the observation that crystalline progesterone had only a slight capacity to maintain spermatogenesis (2.5 mgm. of progesterone daily was about equivalent in its effect to 0.5 mgm. of testosterone). However, the point to be emphasized is that both progesterone and progestin extracts which contain epi-*allo-pregnane-ol-one* are effective in maintaining the testis, yet neither had more than a very slight effect on the sex-accessories or the scrota.

The various experiments cited above have convinced us that the influence of the male sex-hormone on the scrotum plays a relatively minor role in the continuation of spermatogenesis in hypophysectomized animals which have received treatment with androgenic or related substances. The explanation for the maintenance of spermatogenesis, therefore, remains somewhat obscure. It is quite possible, of course, that the simplest explanation possible is the true one, viz., that the spermatogenic elements are directly stimulated by the treatment. In the absence of a more likely theory this is tentatively accepted. The major difficulty attached to its acceptance is the fact that attempts to revive spermatogenesis with male sex hormone have failed. This may, of course, be accomplished with hypophyseal gonadotropic hormone.

Although no definite explanation for the phenomenon we have discussed can be offered at this time the experiments are being continued along several different lines of attack. It is hoped that some satisfactory answer to this very puzzling action of the male sex hormone upon the organ that produces it may be forthcoming from these studies.

SUMMARY

The material discussed in this paper may be summarized briefly. The normal function of the testis depends upon several factors. Among these are the hypophyseal gonadotropic hormones, normal function of the thyroid, and certain vitamins. The testis has two functions, viz., production of sperm and of at least one hormone. The endocrine activity of the testis is concerned with the stimulation of normal function in the sex-accessory structures and with the control of hypophyseal activity. As these effects may all be produced by a single hormone it is unnecessary to postulate the existence of a water-soluble hormone produced by the seminiferous tubules.

Spermatogenesis is seriously impaired by subjection of the testis to confinement in the abdomen. It has been commonly supposed that the endocrine function of the cryptorchid testis is normal. However, it was shown that, in the rat, the production of male hormone is reduced to a very low level if the reproductive organs of animals bearing cryptorchid testes for more than eight months are studied.

Although there can be little doubt that hormones produced by the anterior hypophysis are of primary importance for normal testicular function, evidence was presented which strongly urges an important relation of the testicular hormone to the spermatogenic function of the testis. Sperm production may continue for at least 60 days after hypophysectomy if the animals are treated with preparations of male sex hormone. Such males not only have functional seminiferous tubules, but will mate with normal females and have sired normal litters.

REFERENCES

- Berthold, E. 1849. Transplantation der Hoden. Arch. f. Anat. u. Physiol. u. wissenschaft. Med., p. 42.
Cutuly, E., D. R. McCullagh and E. C. Cutuly. 1937. Effects of androgenic substances in hypophysectomized rats. Am. J. Physiol., 119, 121.
Domm, L. V., R. G. Gustavson and M. Juhn. 1932. Plumage tests in birds. Sex and Internal Secretions (edited by E. Allen), p. 584.
Hamilton, J. B. 1936. Endocrine control of the scrotum and a "sexual skin" in the male rat. Proc. Soc. Exp. Biol. & Med., 35, 386.
Koch, F. C. 1932. The Biochemistry and assay of the testis hormones. Sex and Internal Secretions (edited by E. Allen), p. 372.
Koch, F. C. 1937. The Male sex hormones. Physiol. Rev., 17, 153.
McCullagh, D. R. 1932. Dual endocrine activity of the testis. Science, 76, 19.
Moore, C. R. 1932. The Biology of the testis. Sex and Internal Secretions (edited by E. Allen), p. 281.
Mottram, J. C., and W. Kramer. 1923. On the general effects of exposure to radium on metabolism and tumour growth in the rat and the special effects on testis and pituitary. Quart. J. Exp. Physiol., 13, 209.

- Nelson, W. O., and T. F. Gallagher.** 1936. Some effects of androgenic substances in the rat. *Science*, 84, 230.
- Walsh, E. L., W. K. Cuyler and D. R. McCullagh.** 1934. The physiologic maintenance of the male sex glands. The effect of androtin on hypophysectomized rats. *Am. J. Physiol.*, 107, 508.
- Willier, B. J.** 1932. The embryological foundations of sex in vertebrates. *Sex and Internal Secretions* (edited by E. Allen), p. 94.
- Witschi, E.** 1932. Sex deviations, inversions, and parabiosis. *Sex and Internal Secretions* (edited by E. Allen). p. 160.
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